REMARKS

Any fees that may be due in connection with the filing of this paper or with this application may be charged to Deposit Account No. 06-1050. If a Petition for extension of time is needed, this paper is to be considered such Petition.

Claims 1-49, 51-55, 58-60, 63-76, 88-124, 127-143 and 145-147 are pending. Claims 1 and 124 are amended for clarity. No new matter is added.

I. THE REJECTIONS OF CLAIMS 1-17, 19-27, 29-39, 43-49, 51-54, 58-60, 63-70, 73-76, 86, 88-124 AND 127-145 UNDER 35 U.S.C. §103(a)

Claims 1-17, 19-27, 29-33, 35-37, 43-49, 51, 52, 54, 64-70, 73-76, 124 and 127 are rejected under 35 U.S.C. §103(a) over Köster (WO 94/16101) in view of Cantor (US 5,503,980) on page 2 of the Office Action, claim 34 is rejected under 35 U.S.C. §103(a) over Köster (WO 94/16101) in view of Cantor (US 5,503,980) on page 9 of the Office Action, and claims 38, 39, 53, 58-60, 63, 86, 88, 89-124 and 128-145 are rejected under 35 U.S.C. §103(a) over Köster (WO 94/16101) in view of Cantor (US 5,503,980) on page 11 of the Office Action. The rejections are maintained in the Advisory Action, mailed April 17, 2007.

The Examiner has set forth two bases for rejecting the instant claims under 35 U.S.C. §103(a) over Köster in view of Cantor. In the Advisory Action, mailed April 17, 2007, the Examiner alleges that Köster teaches sequencing using mass labels, and Cantor teaches probe hybridization and sequence determination. The Examiner takes the position that it would have been obvious for one of ordinary skill in the art to use the concept of mass labels as taught by Köster with the concept of sequence determination by hybridization of nucleic acids to an array as described by Cantor, and that the combination results in the instantly claimed methods. The Examiner states that Köster is relied upon for the teaching of using mass labels for sequencing and that Cantor is relied upon for the teaching of probe hybridization.

In the Office Action, mailed February 6, 2007, the Examiner alleges that combining the array of probes of Cantor with the method of sequencing taught by Köster results in the instant method claims. The Examiner urges that Köster teaches a method for sequencing that includes: fragmenting the target nucleic acid, hybridizing the fragment to a target array, where the array contains a collection of probes with sufficient sequence diversity in the variable region to hybridize all target sequences for complete discrimination; determining the molecular weights in the target array to identify hybridized probes, and determining the sequence of the target based upon the hybridized probes. The Examiner states that Cantor teaches an array and a probe with a single-stranded variable region and an array containing the probes. The

Examiner concludes that combining the teachings of Köster with Cantor results in the instantly claimed methods. This rejection respectfully is traversed.

As set forth below, Köster does not teach a method that includes the steps recited by the Examiner. As discussed previously and reiterated below, Köster teaches a method of Sanger sequencing in which the nested molecular weights of the nested fragments are determined by mass spectrometry. By aligning the nested fragments based upon molecular weights, the sequence can be deduced. Köster does not teach positional sequencing by hybridization. The arrays described by Köster present an array of single-stranded nucleic acid molecules or other linker molecules designed to immobilize the nested fragments for presentation in the mass spectrometer. The array is not hybridized to the target sequences, nor does the array include probes; the array includes molecules that can capture the nested sequences. Furthermore, the nested sequences are **not identified** by "mass labels." Mass modifications are employed in multiplex methods in which the sequences of a plurality of target nucleic acid molecules are simultaneously determined. Mass modified bases are used in sequencing reactions to change the molecular weight of nested fragments, permitting assignment of sequence based on molecular weight shift. No where in Köster, is the detection of hybridized probes based on molecular weight taught, disclosed or suggested.

Cantor does teach positional sequencing by hybridization, but does not teach or suggest identifying hybrids by their molecular weight. In the methods Cantor, labels are used to detect hybrids. Cantor does not teach or suggest using mass spectrometry **in place of labels** to identify hybridized probes. Therefore, the combination of teachings of Köster and Cantor does not result in the instantly claimed methods (or arrays). The combination of teachings fails to teach or suggest detecting hybridized probes based upon molecular weight.

With respect to the arrays, Cantor does not teach or suggest detecting hybridized probes in the array by molecular weight using mass spectrometry. As discussed, Köster does not teach arrays of probes. Consequently, the combination of teachings does not result in an array of Cantor that contains matrix material for mass spectrometry and probes with mass modifications as required by claim 124.

RELEVANT LAW

Addressing obviousness, in order to set forth a *prima facie* case of obviousness under 35 U.S.C. § 103:

(1) there must be some teaching, suggestion or incentive supporting the combination of cited references to produce the claimed invention (ACS Hospital Systems, Inc. v. Montefiore Hospital, 732 F.2d 1572, 1577, 221 USPQ 329, 933 (Fed. Cir. 1984)) and

(2) the combination of the cited references must actually teach or suggest the claimed invention.

Further, that which is within the capabilities of one skilled in the art is not synonymous with that which is obvious. Ex parte Gerlach, 212 USPQ 471 (Bd. APP. 1980). Obviousness is tested by "what the combined teachings of the references would have suggested to those of ordinary skill in the art." In re Keller, 642 F.2d 413, 425, 208 USPQ 871, 881 (CCPA 1981), but it cannot be established by combining the teachings of the prior art to produce the claimed invention, absent some teaching or suggestion supporting the combination (ACS Hosp. Systems, Inc. v Montefiore Hosp., 732 F.2d 1572, 1577. 221 USPQ 329, 933 (Fed. Cir. 1984)). "To imbue one of ordinary skill in the art with knowledge of the invention in suit, when no prior art reference or references of record convey or suggest that knowledge, is to fall victim to the insidious effect of a hindsight syndrome wherein that which only the inventor taught is used against its teacher." W.L. Gore & Associates, Inc. v. Garlock Inc., 721 F.2d 1540, 1553, 220 USPQ 303, 312-13 (Fed. Cir. 1983).

While the KSR Court rejected a rigid application of the teaching, suggestion, or motivation ("TSM") test in an obviousness inquiry, the Court acknowledged the importance of identifying "a reason that would have prompted a person of ordinary skill in the relevant field to combine the elements in the way the claimed new invention does" in an obviousness determination. KSR, 127 S. Ct. at 1731. The Court indicated that as long as the TSM test is not applied as a "rigid and mandatory" formula, that test can provide "helpful insight" to an obviousness inquiry. *Id.* Thus, it remains necessary to identify some reason that would have led a the ordinarily skilled artisan to do that which applicant has done. The mere fact that prior art may be modified to produce the claimed product does not make the modification obvious unless the prior art suggests the desirability of the modification. In re Fritch, 23 U.S.P.Q.2d 1780 (Fed. Cir. 1992); see, also, In re Papesh, 315 F.2d 381, 137 U.S.P.Q. 43 (CCPA 1963). Further, that which is within the capabilities of one skilled in the art is not synonymous with that which is obvious. Ex parte Gerlach, 212 USPQ 471 (Bd. App. 1980).

For *prima facie* obviousness to be established under 35 U.S.C. §103, all the claim limitations must be taught or suggested by the prior art. In re Royka, 490 F.2d 981, 180 USPQ 580 (CCPA 1974). This principle of U.S. law regarding obviousness was not altered by the recent Supreme Court holding in KSR International Co. v. Teleflex Inc., 127 S.Ct. 1727, 82

USPQ2d 1385 (2007). In this instance, the combination of teachings of the cited references fails to teach or suggest a method of sequencing in which hybrids are identified by their molecular weight.

THE CLAIMS

Methods for sequencing

Claim1 recites a method for sequencing a target nucleic acid molecule, which includes the steps of fragmenting the target nucleic acid molecule to produce a set of nucleic acid fragments each containing a sequence that corresponds to a sequence of the target nucleic acid; hybridizing the set to an array of nucleic acid probes to form a target array of nucleic acid molecules; **determining molecular weights of hybridized nucleic acids in the target array to identify hybridized probes**; and, based upon the identity of the hybridized probes, determining the sequence of the target nucleic acid. Each probe contains a single-stranded portion that includes a variable region such that each member of the set hybridizes to a member of the array of probes so that the array contains a collection of probes with sufficient sequence diversity in the variable regions to hybridize all of the target sequence with complete or nearly complete discrimination. Claims 2-17, 19-27, 29-33, 35-39, 43-54, 58-60, 63-70, 73-76, 88-123, 128, 145 and 146 ultimately depend from claim 1 and are directed to various embodiments thereof.

Arrays and Systems

Claim 124 recites an array of nucleic acid probes, where each probe includes a single-stranded portion and a constant double-stranded portion; each single-stranded portion includes a variable sequence; the array of probes has sufficient sequence diversity in the variable regions to hybridize to all of a target nucleic acid molecule with complete or nearly complete discrimination; the array is attached to a solid support that includes matrix material that facilitates the volatilization of nucleic acids for mass spectrometry; and the array includes a nucleic acid probe having at least one mass-modifying functionality that increases the discrimination between at least two nucleic acid molecules when detected by mass spectrometry. Claims 129-143 and 147 ultimately depend from claim 124 and are directed to various embodiments thereof.

Claim 127 recites a system that includes the array of claim 124, a mass spectrometer and a computer.

TEACHINGS OF THE CITED ART AND DIFFERENCES FROM THE CLAIMS

Köster (WO 94/16101)

Köster teaches the use of mass spectrometry to analyze the products of Sanger sequencing. In Sanger sequencing, four families of chain-terminated fragments are obtained. The mass difference per nucleotide addition is known and is unique for each of the four nucleotides so that the mass difference between two sequential fragments is indicative of the identity of the added nucleotide. Sanger sequencing generates a set of single stranded nested fragments of a target nucleic acid. Mass spectrometry is employed to analyze the individual single-stranded nested fragments via their different molecular masses. Köster teaches that comparison of the mass difference measured between the nested fragments with the known masses of each chain-terminating nucleotide allows the sequence of each fragment to be determined. Based upon the mass of the fragments, the fragments are aligned and the sequences are determined. In the general nucleic acid sequencing methods in Köster, the molecular weights of a series of nucleic acid fragments of different lengths, which are all subsequences of a single larger sequence, are determined by mass spectrometry. The fragments are identical throughout the portions in which they are the same length. Thus, there is redundancy in the information that is provided by the Sanger method that is used in the methods of Köster. The process of Sanger sequencing is a comparative analysis of recurring information, and in the method of Köster, the fragments are compared to each other and aligned by increasing molecular weight in order to determine the nucleotide sequence. Comparison of the mass difference measured between fragments with the known masses of each chain terminating nucleotide allows the assignment of sequence to be performed.

With respect to mass labels or mass modification, modified nucleotide bases are employed that have different molecules weights from unmodified bases. They are used not for detection, but to permit multiplex sequencing of several targets simultaneously. Modified bases are used in the sequencing reaction of a second target, so that fragments will be uniquely identifiable as derived from that target based on molecular weight. Thus, use of mass modification permits multiplexing. Mass modifications are not required nor specified in the instant independent claims.

Köster does not teach or suggest fragmenting a target nucleic acid molecule and hybridizing the fragments to probes that include a variable single stranded region. In the method of Köster, the single stranded fragments are not captured by probes that contain a variable region such that each member of the set of fragments of a target hybridizes to a member of the array.

Köster does not teach nor suggest capturing fragments using a collection of probes with sufficient sequence diversity in the variable regions to hybridize all of the target sequence with complete or nearly complete discrimination as required by the instant claims. Köster describes using arrays of linkers for immobilizing the nested fragments.

In the capture method of Köster noted by the Examiner, **a linker** is attached to the primer for Sanger sequencing. After producing the nested fragments from the primer, each fragment has a linker L attached. The linker then can be captured by a solid support that contains a complementary linker, L' that is on the solid support. Capture is effected for purification or conditioning of the single-stranded nested fragments prior to analysis. The L-L' linkage is cleaved under the conditions of mass spectroscopy, releasing single-stranded fragments and the molecular weights of the released fragments are determined by mass spectrometry. Köster does not teach or suggest determining the molecular weight of hybridized probes. The method of Köster does not include detection of hybrids nor deduction of a sequence based upon the probe to which a target hybridizes.

As noted, mass modification is used in multiplex methods, in which the sequences of several targets are determined simultaneously. The masses of nucleotide bases of one or more of the targets is/are modified so that the mass difference among the nested fragments will be unique to each target. Thus, in the methods of Köster where mass modification is used, it is the target molecule that contains mass modified bases; Köster does not teach or suggest mass modification of the capture probes.

Hence the sequence methods of Köster differ from the instantly claimed methods in a variety of aspects, including the following:

- Köster does not teach or suggest a probe that includes a single-stranded variable region;
- Köster does not teach or suggest an array of probes, nor an array of probes that includes probes having a single-stranded variable region with sufficient sequence diversity in the variable regions to hybridize all of the target sequence with complete or nearly complete discrimination;
- Köster does not teach or suggest hybridizing fragmented nucleic acid molecules to an array of probes containing a variable region to form a target array;
- Köster does not teach or suggest determining the molecular weight of hybridized probes of an array, nor does the reference teach or suggest determining molecular weights of hybridized nucleic acids in such a target array to identify hybridized probes; and
- Köster does not teach or suggest determining the sequence of the target nucleic acid based upon the identified hybridized probes.

Cantor (U.S. Patent 5,503,980)

Cantor does not teach the deficiencies in the teachings of Köster. Cantor does not teach or suggest identifying hybridized probes in its method by their molecular weights. Cantor teaches a method of positional sequencing by hybridization (PSBH) for determining a nucleotide sequence (col. 7, lines 63 through col. 8, line 6). Positional sequencing by hybridization is a version of DNA sequencing by hybridization (SBH) that uses duplex probes containing single-stranded overhangs, where stacking interactions between the duplex probe and the single-stranded target provides enhanced stringency in distinguishing perfectly matched sequences (col. 3, lines 29-41). The method of Cantor relies upon **labeling the target nucleic acid and detecting the label for identification of target nucleic acid** that hybridizes to the probes. In the method of Cantor, positional information can be determined based upon determining the location of label, such as using the ratio of internal label to terminal label.

There is no teaching or suggestion in Cantor for identifying hybridized probes in an array by determining the molecular weights of hybridized nucleic acid in the target array. The only teachings directed to molecular weight in Cantor are the teaching in Example 2 directed to fractionation of a sample, and reference to the Maxim and Gilbert sequencing technique, where terminally labeled DNA molecules are chemically cleaved at single base repetitions and then the molecular weight of each partially cleaved fragment is determined using electrophoresis to produce a pattern of fragments on a gel, whereby the DNA sequence can be read (see col. 1, lines 24-35). Hence, Cantor does not teach or suggest determining molecular weights of hybridized nucleic acids in array of probes in order to identify hybridized probes, and based on the identity of the hybridized probes, determining the sequence of the target nucleic acid.

ANALYSIS

It is respectfully submitted that the Examiner has failed to set forth a case of *prima facie* obviousness because the **combination of teachings of does not result** in the instantly claimed methods or arrays.

Combination of teachings of Koster and Cantor

1. Methods

The instant method claims include as steps determining molecular weights of hybridized nucleic acids in a target array to identify hybridized probes, and based upon the identified hybridized probes, determining the sequence of the target nucleic acid.

As discussed above, Köster does not teach or suggest identifying hybridized probes in an array based upon molecular weight, and based upon the identified hybridized probes, determining the sequence of the target nucleic acid.

Cantor does not cure this deficiency. Cantor teaches positional sequencing by hybridization, which requires labeling the target nucleic acid and detecting the label. There is no teaching or suggestion in Cantor for identifying hybridized probes in an array by determining the molecular weights of hybridized nucleic acid in the target array. Cantor does not teach or suggest determining molecular weights of hybridized nucleic acids in the target array to identify hybridized probes, and based upon the hybridized probes, determining the sequence of the target nucleic acid, which is an element of the instant claims. Hence, Cantor does not teach or suggest subject matter of the claims missing from Köster. Therefore, the combination of teachings of Köster and Cantor fails to teach or suggest a method of sequencing in which the hybridized probes are identified by molecular weight. Therefore, the Examiner has failed to set forth a *prima facie* case of obviousness of any of claims 1 2-17, 19-27, 29-33, 35-39, 43-54, 58-60, 63-70, 73-76, 88-123 and 128 ultimately depend from claim 1 and include the limitations thereof.

2. Arrays and Systems

In the instantly claimed arrays, the probes are provided on a solid support that includes a matrix chemical that facilitates the volatilization of nucleic acids for mass spectrometry. The probes include at least one mass-modifying functionality that increases the discrimination between the nucleic acid probe with the mass modifying functionality and another nucleic acid molecule.

Köster does not teach or suggest an array of **probes for SBH or PSBH and further does not suggest** including at least one mass-modifying functionality that increases the discrimination between the nucleic acid probe with the mass modifying functionality and another nucleic acid molecule when detected by mass spectrometry in such a array. In Köster, mass modifications may be introduced into the nested nucleic acid fragments of the target via an oligonucleotide primer, chain-terminating nucleoside triphosphates and/or chain-elongating nucleoside triphosphates for discrimination when using multiplexing detection. Köster does not teach or suggest mass-modifying probes of an array such that the array includes a nucleic acid probe having at least one mass-modifying functionality that increases the discrimination between the nucleic acid probe with the mass modifying functionality and another nucleic acid

molecule. Any arrays include the immobilized nested fragments, not probes for PSBH nor hybridized probes for PSBH.

As discussed, Cantor teaches arrays of probes for PSBH. Cantor does not teach or suggest including mass modifications in any of the probes and/or including matrix material in the array. Cantor does not teach or suggest the elements missing from Köster. For example, Cantor does not teach or suggest an array of nucleic acid probes that includes a nucleic acid probe having at least one mass-modifying functionality that increases the discrimination between the nucleic acid probe with the mass modifying functionality and another nucleic acid molecule when detected by mass spectrometry. Cantor does not teach or suggest detection by molecular weight. Cantor relies upon labeling the target nucleic acid for detection. There is no teaching or suggestion in Cantor of identifying hybridized probes in an array by their molecular weights. Accordingly, there is no teaching in Cantor to include in its arrays a nucleic acid probe having at least one mass-modifying functionality that increases the discrimination between the nucleic acid probe with the mass modifying functionality and another nucleic acid molecule when detected by mass spectrometry. Hence, Cantor does not teach or suggest all the elements missing from the teaching of Köster. Combining the teachings of Köster and Cantor does not result in an array of probes for PSBH that includes mass modifications in the probes and matrix material in the array. Thus, the combination of teachings of Köster and Cantor does not result in the array of nucleic acid probes of claim 124 nor any claims dependent thereon. Therefore, the Examiner has failed to set forth a *prima facie case* of obviousness of claims 124, 127, 129 and 144-147.

REBUTTAL TO THE EXAMINER'S COMMENTS

Rejection of Claims 1-17, 19-27, 29-33, 35-39, 43-54, 58-60, 63-70, 73-76, 88-123, 128, 145 and 146 – Methods for sequencing a target nucleic acid molecule

1. Labels

In the Advisory Action, the Examiner states that Köster is relied upon for the teaching of using mass labels for sequencing and that Cantor is relied upon for the teaching of probe hybridization. The Examiner alleges that it would have been obvious to use the concept of mass labels as taught by Köster with the concept of sequence determination by hybridization of nucleic acids to an array as described by Cantor.

Applicant respectfully submits that the methods of sequencing taught in Köster do not rely on "mass labels." Köster teaches determining the molecular weight of individual Sanger fragments, and that comparison of the mass difference measured between the nested fragments

with the known masses of each chain-terminating nucleotide allows the sequence of each fragment to be determined. Based upon the mass of the fragments, the fragments are aligned and the sequence of the target nucleic acid molecule is determined. No "mass labels" are used for determining the sequence – a nested set of fragmented target nucleic acid is produced and the molecular weights of the single-stranded fragments is determined directly. The method of sequencing of Köster does not rely on the detection of a label. The only discussion in Köster regarding using labels is with respect to the prior art methods, where Köster describes the use of labels as creating several problems, such as posing a risk to health, being laborious and difficult to automate and reducing discriminating power (see pages 3-8). Köster teaches that mass spectroscopy obviates the need for substitutions with detectable labels such as isotopes (see page 9, lines 19-23). Hence, Köster actually teaches away from using detectable labels.

It appears that the Examiner misunderstands what is meant by "mass modification" as taught in Köster. It is **not** a label. Köster teaches that a mass modification can be used to discriminate between several samples of nucleic acid that are pooled together and analyzed at once, such as in multiplex mass spectrometry (*e.g.*, see page 18, lines 1-8). In order to distinguish among the source of the several fragments in multiplexing analysis, a different mass modification that produces a known molecular ion peak upon analysis is introduced into the different target nucleic acid molecules, such as by using mass-modified nucleic acid primers or mass-modified nucleoside triphosphates. This results in a family of fragments that include a distinguishable mass modification. The different mass-modified fragments produce different mass peaks that can be distinguished from fragments of another target nucleic acid molecule, and fragment families can be separated by identifying the known molecular ion peaks of the selected mass modification of each target family (page 20, lines 4-39). The mass modification provides a means of discriminating between fragment families because fragments from the same family will have the same mass increment, which is different from the mass increments of the other fragment families (e.g., see page 21, lines 30-39). In the methods of Köster that include multiplexing, the mass modification itself is not detected for sequencing. The molecular weight of each of the pooled fragments is determined for sequencing. In the methods of Köster using multiplexing, the pooled fragments are separated into fragment families based on mass modification, because fragments from the same family will have the same mass increment. The sequence of each target nucleic acid molecule then is determined based on the molecular weight of the fragments.

Applicant respectfully submits that the instant methods do not rely on using mass labels for sequencing. Claim 1 and its dependent claims recite methods of sequencing a target

nucleic acid molecule that include as a step determining molecular weights of hybridized nucleic acids in the array to identify hybridized probes. Claim 1 and its dependent claims recite that the method of determining the sequence of the target nucleic acid is based upon the identified hybridized probes, not on detection of any type of label.

The instant specification teaches that mass modification can be used to provide a distinction detectable by mass spectrometry to distinguish between two or more reaction products. The instant specification teaches that mass modification that increases the discrimination between at least two nucleic acids can be used to facilitate sequencing. Such mass modifications can be used in the instantly claimed methods, such as when using multiplexing mass spectrometry, but as discussed above, such mass modifications are not labels used for sequencing nor are they required in the instant methods. Mass modification provides a means of distinguishing between two or more reaction products, such as extension products of different target nucleic acid molecules.

The instant methods do not rely on detection of any type of label for sequencing. Instead, the instant methods of determining the sequence of a target nucleic acid includes as an element identifying hybridized probes in the array by determining molecular weights of hybridized nucleic acids in the target array to identify hybridized probes, and based upon the identified hybridized probes, determining the sequence of the target nucleic acid.

Thus, the premise stated in the Advisory Action on which the rejection is based contains errors. Köster does not teach using mass labels for sequencing, and actually teaches away from using detectable labels. Further, the instantly claimed methods do not rely on the use of any type of labels for sequencing. Furthermore, as set forth above, the combination of teachings of Koster and Cantor do not result in the instantly claimed methods or arrays.

2. Combining the Methods of Köster with the Probes of Cantor

In the Office Action, the Examiner alleges that it would have been obvious to combine the probes with sequence diversity in the variable regions as taught by Cantor with the method of sequencing nucleic acid by mass spectrometry as taught by Köster in order to achieve the expected advantage of enhanced sequence stringency and more accurate sequencing of the target DNA, and that the combination of Köster and Cantor results in the instant methods. Applicant respectfully submits that, for the reasons discussed previously and below, combining the teachings of Köster and Cantor does not result in the instantly claimed methods.

As discussed above, Köster teaches using Sanger sequencing to generate a set of single-stranded nested fragments of a target nucleic acid and using mass spectrometry to analyze the

individual single-stranded nested fragments via their different molecular masses. Köster teaches that comparison of the mass difference measured between the nested fragments with the known masses of each chain-terminating nucleotide allows the sequence of each fragment to be determined. Comparing the mass difference measured between fragments, each of which differs by its chain-terminating nucleotide, with the known masses of each chain terminating nucleotide, allows the fragments to be aligned and the sequence to be assigned.

In contrast, the instantly claimed methods do not rely on Sanger sequencing or production of a nested set of nucleotide fragments that differ only in the chain-terminating nucleotide or alignment of a nested set of fragments for sequencing. In the instant methods, a target nucleic acid molecule is fragmented to produce a set of nucleic acid fragments each having a portion of the target nucleic acid. These fragments are hybridized to an array of nucleic acid probes, each of which includes a single-stranded variable region. The molecular weight of the hybridized probes is measured to identify probes that have formed hybrids. Based on the identified probes, the sequence is determined.

Hence, the instant methods are very different from the Sanger sequencing method of Köster in which sets of nested fragments that differ from each other by their chain-terminating nucleotide are produced and the relative molecular weights of the individual single-stranded fragments determined by mass spectrometry. Köster does not teach or suggest determining the sequence of a target nucleic acid by identifying hybridized probes in an array by determining the molecular weight of the hybridized probes, and based on the identified probes, determining the sequence of the target nucleic acid. Thus, the method of Köster has nothing to do with sequencing by hybridization nor identifying hybridized probes in an array based upon molecular weight.

The method of positional sequencing by hybridization of Cantor relies upon labeling the target nucleic acid and detecting the label. In the methods of Cantor, positional information can be determined using the ratio of internal label to terminal label. For example, positional information about the distance between a known 3'-terminal sequence and a known reference point could be obtained by using nested targets with a common labeled 5' end and variable 3' ends, where the fragments include a second internal label. In the method of Cantor, the positional information gleaned from the position of the labels, such as from an internal label compared to a 5' label, is helpful in reconstructing the DNA sequence and can potentially be used to resolved ambiguities, such as those that could be caused by interspersed repeated sequences. There is no teaching or suggestion in Cantor of identifying hybridized probes in an

array by determining the molecular weights of hybridized nucleic acid in the target array. Cantor does not teach or suggest determining molecular weights of hybridized nucleic acids in the target array to identify hybridized probes, and based upon the identified hybridized probes, determining the sequence of the target nucleic acid, which is an element of the instant claims. As discussed above, Köster does not teach or suggest determining molecular weights of hybridized nucleic acids in the target array formed by hybridization of nucleic acid to probes in the array to identify hybridized probes, and based upon the identified hybridized probes, determining the sequence of the target nucleic acid. Hence, combining the probes of Cantor with the methods of sequencing taught in Köster does not result in the instantly claimed methods.

II. THE REJECTION OF CLAIM 28 UNDER 35 U.S.C. §103(a)

Claim 28 is rejected under 35 U.S.C. §103(a) over Köster and Cantor in view of Weiss (U.S. 6,025,193) because the combination of Köster and Cantor allegedly teaches all elements of claim 28 except generation of thiol moieties by using Beucage reagent, but Weiss allegedly cures this deficiency. This rejection is respectfully traversed.

RELEVANT LAW

See related section above.

CLAIM 28

Claim 28 ultimately depends from claim 1 and is directed to an embodiment where a mass-modifying functionality is a thiol moiety generated by using Beucage reagent.

TEACHINGS OF THE CITED ART AND DIFFERENCES FROM THE CLAIMS

Köster and Cantor

The teachings of Köster and Cantor are discussed above.

Weiss (U.S. 6,025,193)

Weiss teaches methods and compositions for diagnosing and treating pathological conditions related to a dopamine receptor abnormality. The reference teaches that unmodified oligodeoxynucleotides can be converted into phosphorothioate oligodeoxynucleotides by replacing iodine used for standard oxidation with Beucage reagent. Weiss teaches that using Beucage reagent results in the replacement of every oxygen group of the phosphodiester bond with a sulfur group, and that such substitutions result in an asymmetric distribution of the negative charge to predominate on the sulfur atom, resulting in "improved stability to nucleases, retention of solubility in water and stability to base-catalyzed hydrolysis" (col. 13, lines 2-14) and improved *in vivo* stability (col. 15, lines 41-45).

Weiss does not teach or suggest a probe that includes a single-stranded variable region or an array of probes. Weiss does not teach or suggest an array of probes that includes probes having a single-stranded variable region with sufficient sequence diversity in the variable regions to hybridize all of the target sequence with complete or nearly complete discrimination. Weiss does not teach or suggest hybridizing fragmented nucleic acid molecules to an array of probes containing a variable region to form a target array. Weiss does not teach or suggest determining the molecular weight of hybridized probes of an array, nor does the reference teach or suggest determining molecular weights of hybridized nucleic acids in such a target array to identify hybridized probes. Weiss does not teach or suggest identifying hybridized probes in an array based upon molecular weight, and based upon the identified hybridized probes, determining the sequence of the target nucleic acid.

ANALYSIS

It is respectfully submitted that the Examiner has failed to set forth a case of *prima facie* obviousness for the following reasons.

The combination of the teachings of Köster and Cantor with the teachings of Weiss does not result in the instantly claimed methods.

As discussed above, the combination of the teachings of Köster and Cantor does not teach or suggest a method for sequencing a target nucleic acid that includes as elements identifying hybridized probes in the array by determining the molecular weight of the hybridized probes, and based on the identified hybridized probes, determining the sequence of the target nucleic acid. Claim 28 depends from claim 1 and includes every limitation thereof. Accordingly, the combination of the teachings of Köster and Cantor does not teach or suggest every element of claim 28.

Weiss does not teach or suggest the subject matter missing from the combination of the teachings of Köster and Cantor. Weiss does not teach or suggest identifying hybridized probes in an array by determining the molecular weight of the hybridized probes, and based on the identity of the hybridized probes, determining the sequence of the target nucleic acid. Thus, even if Weiss teaches generating thiol moieties using Beucage reagent, Weiss fails to cure the deficiencies in the combination of the teachings of Köster and Cantor because Weiss does not teach or suggest the elements of the claimed subject matter missing from the combination of the teachings of Köster and Cantor.

None of Köster, Cantor nor Weiss, individually nor in any combination, teaches or suggests a method for sequencing a target nucleic acid that includes as an element determining

molecular weights of hybridized nucleic acids in the target array to identify hybridized probes, and based on the identified hybridized probes, determining the sequence of the target nucleic acid. Thus, combining the teachings of Köster and Cantor with the teachings of Weiss does not result in the instantly claimed method of claim 28. Therefore, the Examiner has failed to set forth a *prima facie* case of obviousness.

III. THE REJECTION OF CLAIMS 71 AND 72 UNDER 35 U.S.C. §103(a)

Claims 71 and 72 are rejected under 35 U.S.C. §103 as being unpatentable over Köster and Cantor in view of Sanghvi *et al.* (U.S. Patent No. 6,214,551) because the combination of the teachings of Köster and Cantor allegedly teaches all elements of the claims except that the selectively releasable bond is 4,4'-dimethoxy-trityl or a derivative thereof, and Sanghvi *et al.* allegedly cures this deficiency. The Examiner contends that Sanghvi *et al.* teaches the selectively releasable bond 4,4'-dimethoxytrityl or a derivative thereof, and argues that although the reference does not teach the derivative 3 or 4 [bis-(4-methoxy-phenyl)]-methyl-benzoic acid in particular, Sanghvi *et al.* teaches equivalent compounds and derivatives used for the same purpose. This rejection is respectfully traversed.

RELEVANT LAW

See related section above.

CLAIMS 71 AND 72

Claims 71 and 72 ultimately depend from claim 1 and are directed to embodiments thereof. Claim 71 specifies that each probe of the array is attached to the solid support by a selectively releasable bond and comprises 4, 4'-dimethoxytrityl or a derivative thereof. Claim 72 depends from claim 71, and recites that the derivative is selected from among 3 or 4 [bis-(4-methoxyphenyl)]methyl benzoic acid, N-succinimidyl-3 or 4 [bis-(4-methoxyphenyl)]methyl-benzoic acid, N-succinimidyl-3 or 4 [bis-(4-methoxyphenyl)]-hydroxy-methyl-benzoic acid, N-succinimidyl-3 or 4 [bis-(4-methoxyphenyl)]-chloromethyl-benzoic acid and salts thereof.

TEACHINGS OF THE CITED ART AND DIFFERENCES FROM THE CLAIMS

Köster and Cantor

The teachings of Köster and Cantor are discussed above.

Sanghvi *et al.* (U.S. Patent 6,214,551)

Sanghvi *et al.* teaches compounds that mimic and/or modulate the activity of wild-type nucleic acids. Sanghvi *et al.* teaches the use of dimethoxytrityl groups as a blocking group

during nucleoside polymerization. Sanghvi *et al.* teaches that an oligonucleotide is tethered to a solid support via its 3' hydroxyl group (col. 57, line 63 through col. 58, line 14).

Sanghvi *et al.* does not teach or suggest a probe that includes a single-stranded variable region or an array of probes. Sanghvi *et al.* does not teach or suggest an array of probes that includes probes having a single-stranded variable region with sufficient sequence diversity in the variable regions to hybridize all of the target sequence with complete or nearly complete discrimination. Sanghvi *et al.* does not teach or suggest hybridizing fragmented nucleic acid molecules to an array of probes containing a variable region to form a target array. Sanghvi *et al.* does not teach or suggest determining the molecular weight of hybridized probes of an array. Sanghvi *et al.* does not teach or suggest identifying hybridized probes in an array based upon molecular weight, and based upon the identified hybridized probes, determining the sequence of the target nucleic acid.

ANALYSIS

It is respectfully submitted that the Examiner has failed to set forth a case of *prima facie* obviousness for the following reasons.

The combination of the teachings of Köster and Cantor with the teachings of Sanghvi *et al.* does not result in the instantly claimed methods.

Claims 71 and 72 ultimately depend from claim 1 and are directed to embodiments thereof. Thus, claims 71 and 72 include every limitation of claim 1. As discussed above, the combination of the teachings of Köster and Cantor does not teach or suggest methods for sequencing a target nucleic acid that include as an element determining molecular weights of hybridized nucleic acids in the target array to identify hybridized probes, and based on the identified probes, determining the sequence. Sanghvi *et al.* does not cure this deficiency. Sanghvi *et al.* does not teach or suggest sequencing a nucleic acid by hybridizing fragmented target nucleic acid to an array as claimed and determining molecular weights of hybridized nucleic acids in the target array to identify hybridized probes, and based upon the identified hybridized probes, determining the sequence of the target nucleic acid. Hence, Sanghvi *et al.* does not teach or suggest the elements missing from the combined teachings of Köster and Cantor.

Further, Claim 71 is directed to an embodiment where each probe is attached to a solid support by a selectively releasable bond that includes 4, 4'-dimethoxytrityl or a derivative thereof, and claim 72 specifies the derivatives of 4, 4'-dimethoxytrityl. Contrary to the Examiner's allegations, Sanghvi *et al.* does not teach or suggest selectively attaching a nucleic

acid probe to a solid support via releasable bonds containing 4,4'-dimethoxytrityl or a derivative thereof. Sanghvi *et al.* teaches the use of dimethoxytrityl groups as a blocking group during nucleoside polymerization. In Example 81, Sanghvi *et al.* teaches that an oligonucleotide is tethered to a solid support via its 3' hydroxyl group, not via a dimethoxytrityl group. The Examiner cites col. 59, lines 3-32 of Sanghvi *et al.* to support the allegation that the reference teaches 4,4'-dimethoxytrityl as a selectively releasable bond attaching a probe to a solid support. The recited section of Sanghvi *et al.* states:

> The dimeric oligonucleoside 58 will be utilized as building block units in a conventional oligonucleotide solid support synthesis as per the procedure of Example 80. For the purpose of illustration a polymer incorporating seven nucleosides is described. A first unit of the dimeric oligonucleoside 58 will be coupled to a first cytidine nucleoside **tethered to a solid support via its 3' hydroxyl group** and having a free 5' hydroxyl group. After attachment of the first unit of compound 58 to the support, the 5'-dimethoxytrityl group of that first compound 58 unit will be removed in the normal manner. A second compound 58 unit will then be coupled via its β-cyanoethyl-N-diisopropylphosphiryl group to the first compound 58 unit using normal phosphoramidate chemistry. This forms a conventional phosphodiester bond between the first and second compound 58 units and elongates the polymer by two nucleosides (or one oligonucleoside dimer unit). The **dimethoxytrityl blocking group** from the second compound 58 unit will be removed in the normal manner and the polymer elongated by a further dimeric unit of compound 58. As with addition of the first and second dimeric units, the third unit of compound 58 is coupled to the second via conventional phosphoramidite procedures. The addition of the third unit of compound 58 completes the desired length and base sequence. This polymer has a backbone of alternating normal phosphodiester linkages and the methyl-(iminooxymethylene) linkages of compound 58. **The 5' terminal dimethoxytrityl group of the third compound 58 unit will be removed in the normal manner** followed by release of the polymer from the solid support, also in the normal manner. ... [emphasis added]

There is no teaching or suggestion in Sanghvi *et al.* that a dimethoxytrityl group is a selectively reversible bond for attaching a nucleic acid molecule to a solid support. Instead, Sanghvi *et al.* teaches that dimethoxytrityl groups are useful for protecting intermediates during synthesis, especially as a hydroxyl protecting group (see col. 15, lines 8-19). Sanghvi *et al.* does not teach or suggest any of 3 or 4 [bis-(4-methoxyphenyl)]-methyl-benzoic acid, N-succinimidyl-3 or 4 [bis-(4-methoxyphenyl)]-methyl-benzoic acid, N-succinimidyl-3 or 4 [bis-(4-methoxyphenyl)]-hydroxymethyl-benzoic acid, N-succinimidyl-3 or 4 [bis-(4-methoxyphenyl)]-chloromethyl-benzoic acid as a selectively releasable bond for attaching a nucleic acid molecule to a solid support. Thus, the combination of the teachings of Köster and Cantor with the teachings of Sanghvi *et al.* does not teach or suggest all the elements of the methods of claims 71 and 72.

None of Köster, Cantor nor Sanghvi *et al.*, alone or in any combination, teaches or suggests a method for sequencing a target nucleic acid that includes as an element determining molecular weights of hybridized nucleic acids in the target array to identify hybridized probes, and based on the identity of the hybridized probes, determining a sequence. Further, none of Köster, Cantor nor Sanghvi *et al.*, alone or in any combination, teaches or suggests the limitations of claims 71 and 72. Thus, combining the teachings of Köster and Cantor and Sanghvi *et al.* does not result in the instantly claimed methods of claims 71 and 72. Therefore, the Examiner has failed to set forth a *prima facie* case of obviousness.

IV. REBUTTAL TO EXAMINER'S ARGUMENTS

A. Abstract of Köster

In the Advisory Action, the Examiner alleges that the Abstract of Köster teaches determining molecular weights of nucleic acids in the target array to identify hybridized probes and subsequently determining the sequence of the target nucleic acid. Applicant respectfully disagrees. The Abstract of Köster recites:

> The invention describes a new method to sequence DNA. The improvements over the existing DNA sequencing technologies are high speed, high throughput, no electrophoresis and gel reading artifacts due to the complete absence of an electrophoretic step, and no costly reagents involving various substitutions with stable isotopes. The invention utilizes the Sanger sequencing strategy and assembles the sequence information by analysis of the nested fragments obtained by base-specific chain termination via their different molecular masses using mass spectrometry, as for example, MALDI or ES mass spectrometry. A further increase in throughput can be obtained by introducing mass-modifications in the oligonucleotide primer, chain-terminating nucleoside triphosphates and/or in the chain-elongating nucleoside triphosphates, as well as using integrated tag sequences which allow multiplexing by hybridization of tag specific probes with mass-differentiated molecular weights.

Contrary to the Examiner's allegations, there is no teaching or suggestion in the Abstract of Köster of a target array or determining molecular weight to identify hybridized probes or determining a sequence of a target nucleic acid based on the identity of the hybridized probes.

B. Alleged "Attack" of the References in a "Piecemeal" Manner

In maintaining the previous rejections, the Examiner alleges that Applicant's argument "attacked" the references individually and in a "piecemeal" manner instead of addressing the combination of the references, and stated that one cannot show nonobviousness by attacking references individually where the rejections are based on combinations of references.

It is respectfully submitted that Applicant did not attack references individually in rebuttal of the rejections set forth under 35 U.S.C. § 103(a). Rather, Applicant systematically (i) distinguished the teachings of each of the cited references from the instantly claimed subject

matter; and (ii) showed that the deficiencies of each of the cited references against the claimed subject matter was not cured by any of the other cited references (or "routine art"). Applicant combined the teachings to show that the combination of teachings of the references, singly or in any combination, does not teach or suggest the claimed subject matter.

The Examiner is directed to the section at pages 14-16 of the previous Response, filed March 28, 2007, with the header "ANALYSIS" and the header "The combination of the teachings of Köster with the teachings of Cantor does not result in the instantly claimed methods," which discusses how the combination of the cited references does not result in the claimed subject matter because, among other reasons of record in the aforementioned Response, all the cited references lack a teaching or suggestion of the instantly claimed element of identifying hybridized probes in an array by determining the molecular weight of the hybridized probes and based on the identity of the hybridized probes, determining the sequence of the target nucleic acid. For example, the cited section states that Köster teaches using base-specific chain termination (Sanger sequencing) to generate a set of nested fragments of a target nucleic acid and using mass spectrometry to analyze the nested fragments via their different molecular masses. Thus, Köster does not teach or suggest determining molecular weights of hybridized nucleic acid molecules in an array. Instead, Köster teaches determining molecular weights of desorbed single-chain nucleic acid fragments. Köster does not teach or suggest identifying hybridized probes in an array based upon molecular weight of the hybridized nucleic acids, and based on the identified hybridized probes, determining the sequence of the target nucleic acid.

Cantor does not teach the elements missing from Köster. In the method of Cantor, the sequence is determined based upon detecting a label and determining the location of label, such as the determination of positional information using the ratio of internal label to terminal label. Thus, the method of Cantor relies upon labeling the target nucleic acid and detecting the label. The instantly claimed methods do not rely on detecting a label. There is no teaching or suggestion in Cantor of identifying hybridized probes in an array by determining the molecular weights of the hybridized nucleic acids in the target array. Cantor does not teach or suggest determining molecular weights of hybridized nucleic acid in the target array to identify hybridized probes, which is an element of the instant claims. Hence, Cantor does not teach or suggest the subject matter missing from Köster.

Thus, the cited references, singly or in combination thereof, fail to teach or suggest the missing elements of the claims. Therefore, it is respectfully submitted that Applicant rebutted

the obviousness rejections based on the teachings of the combinations of references after systematically distinguishing each of the cited references from the elements of the instant claims and demonstrating that the deficiencies of each of the cited references against the claimed subject matter was not cured by any combination of the cited references.

2. Köster and Cantor and Weiss

Similarly, for the rejection of claim 28 under 35 U.S.C. §103(a) over Köster and Cantor in view of Weiss, the Examiner is directed to the section at pages 18-19 of the previous Response, filed March 28, 2007, with the header "ANALYSIS" and the header "The combination of the teachings of Köster and Cantor with the teachings of Weiss does not result in the instantly claimed methods." Applicant systematically distinguished the teachings of each of the cited references from the instantly claimed subject matter, and showed that the deficiencies of each of the cited references against the claimed subject matter was not cured by any of the other cited references (or "routine art"). Applicant then pointed out that none of the references, singly or in any combination, taught or suggested the claimed subject matter.

For example, the section states that claim 28 ultimately depends from claim 1, is directed to embodiments thereof, and therefore includes every limitation of claim 1. As discussed above, the section states that the combination of the teachings of Köster and Cantor does not teach or suggest methods for sequencing a target nucleic acid that include as an element determining molecular weights of hybridized nucleic acids in the target array to identify hybridized probes, and based on the identity of the hybridized probes, determining the sequence of the target nucleic acid. Weiss does not teach or suggest identifying hybridized probes in an array by determining the molecular weight of the hybridized probes, and based on the hybridized probes, determining the sequence of the target nucleic acid. Thus, even if Weiss teaches generating thiol moieties using Beucage reagent, Weiss fails to cure the deficiencies in the combination of the teachings of Köster and Cantor because Weiss does not teach or suggest the elements of the claimed subject matter missing from the combination of the teachings of Köster and Cantor. None of Köster, Cantor nor Weiss, individually nor in any combination, teaches or suggests a method for sequencing a target nucleic acid that includes as an element determining molecular weights of hybridized nucleic acids in the target array to identify hybridized probes, and based on the hybridized probes, determining the sequence of the target nucleic acid.

Thus, combining the teachings of Köster and Cantor with the teachings of Weiss does not result in the instantly claimed method of claim 28. Therefore, it is respectfully submitted that Applicant rebutted the obviousness rejections based on the teachings of the combinations

of references after distinguishing each of the cited references from the elements of the instant claims and demonstrating that the deficiencies of each of the cited references against the claimed subject matter was not cured by any of the other cited references.

3. Köster and Cantor and Sanghvi *et al.*

Similarly, for the rejection of claims 71 and 72 under 35 U.S.C. §103 as being unpatentable over Köster and Cantor in view of Sanghvi *et al.*, the Examiner is directed to the section at pages 19-21 of the previous Response, filed March 28, 2007, with the header "ANALYSIS" and the header "The combination of the teachings of Köster and Cantor with the teachings of Sanghvi *et al.* does not result in the instantly claimed methods." Applicant systematically distinguished the teachings of each of the cited references from the instantly claimed subject matter and showed that the deficiencies of each of the cited references against the claimed subject matter was not cured by any of the other cited references (or "routine art"). Applicant then pointed out that none of the references, singly or in any combination, taught or suggested the claimed subject matter.

For example, the section states that claims 71 and 72 ultimately depend from claim 1, are directed to embodiments thereof and thus include every limitation of claim 1. As discussed above, the combination of the teachings of Köster and Cantor does not teach or suggest methods for sequencing a target nucleic acid that include as an element determining molecular weights of hybridized nucleic acids in the target array to identify hybridized probes, and based on the identity of the hybridized probes, determining the sequence of the target nucleic acid. Sanghvi *et al.* does not cure this defect. Sanghvi *et al.* does not teach or suggest sequencing a nucleic acid by hybridizing fragmented target nucleic acid to an array as claimed and determining molecular weights of hybridized nucleic acids in the target array to identify hybridized probes. Hence, Sanghvi *et al.* does not teach or suggest the elements missing from the combined teachings of Köster and Cantor.

Thus, the cited references, singly or in any combination thereof, fail to teach or suggest the missing elements of the claims. Therefore, it is respectfully submitted that Applicant rebutted the obviousness rejections based on the teachings of the combinations of references after distinguishing each of the cited references from the elements of the instant claims and demonstrating that the deficiencies of each of the cited references against the claimed subject matter was not cured by any of the other cited references.

* * *

In view of the above remarks and amendment, reconsideration and withdrawal of the rejections and allowance of the application are respectfully requested.

Respectfully submitted,

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